Supplemental Information

SARS-CoV-2 Antibody Responses Correlate with Resolution of RNAemia But Are Short-Lived in Patients with Mild Illness

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Supplementary Figures

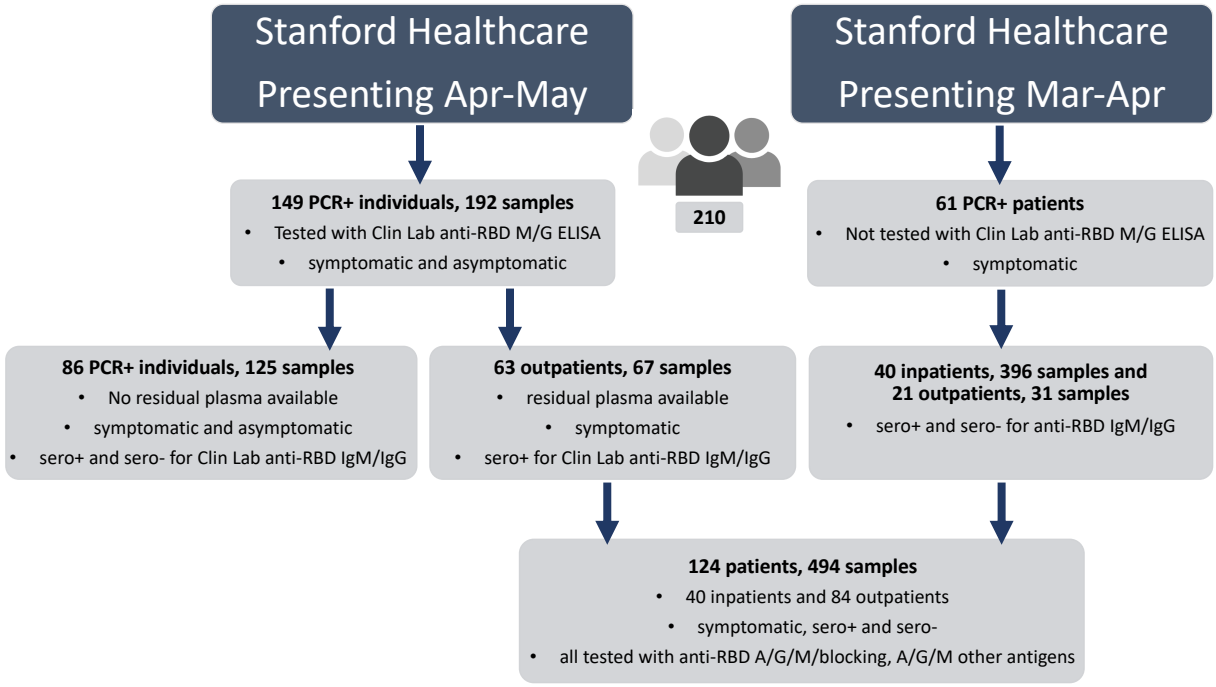

Fig. S1. Study design and participant overview.

210 SARS-CoV-2 rRT-PCR-positive (PCR+) individuals recruited after presenting to Stanford Healthcare were included in the study. 61 patients presented with symptoms of COVID-19 before April 8, 2020, when routine Clinical Laboratory (Clin Lab) testing for the presence of anti-RBD IgM/IgG became available. 149 individuals either presented with symptoms or participated in occupational health screening on or after April 8. Residual serial plasma samples for a detailed study of IgM, IgG, and IgA antibody responses to SARS-CoV-2 proteins and SARS-CoV RBD were available from 124 symptomatic COVID-19 patients.

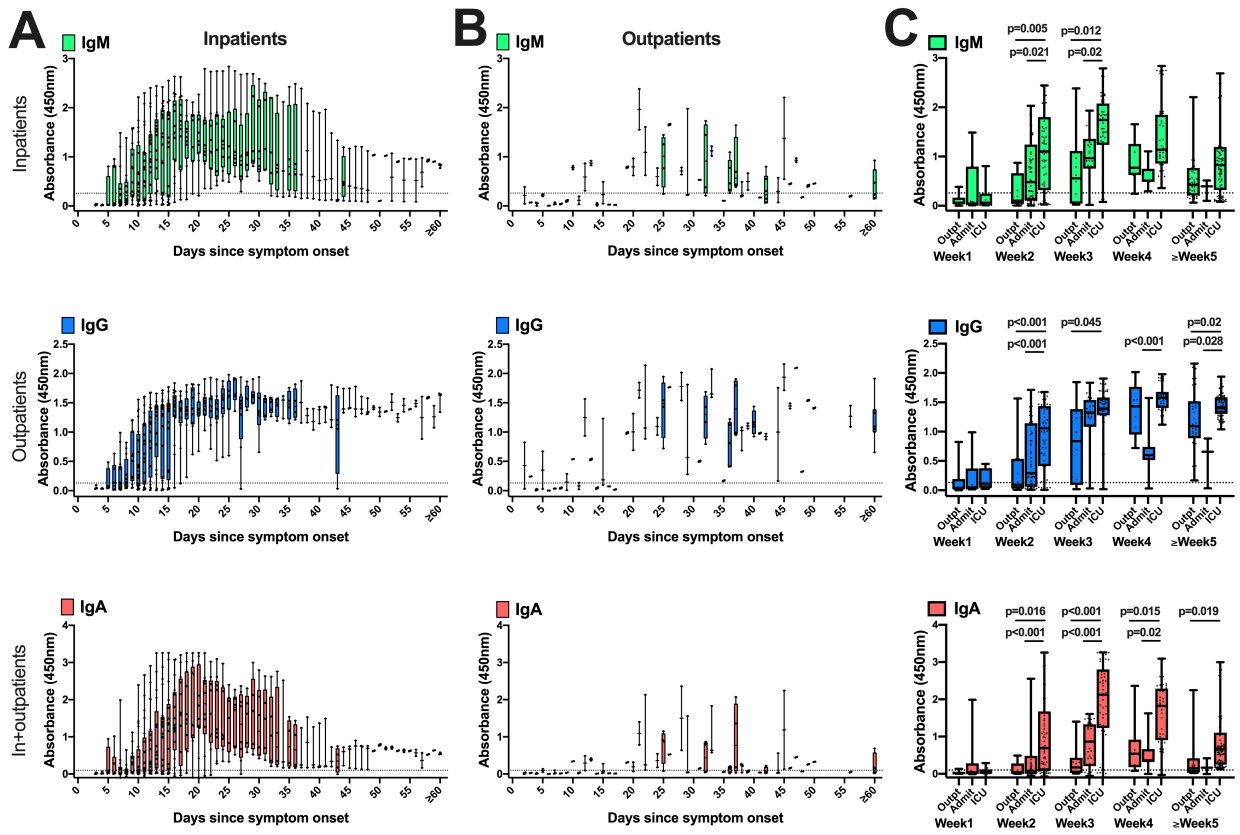

Fig. S2. Development of anti-SARS-CoV-2 spike S1 antibody titers in COVID-19 patients.

494 plasma samples from 124 COVID-19 patients were tested for the presence of virus-specific IgM, IgG, and IgA antibodies. ELISA OD_{450} values are plotted separately for samples from inpatients (**A**) and outpatients (**B**). Box-whisker plots show the interquartile range as the box and the minimum and maximum values as the ends of the whiskers. Data stratified by outpatients (Outpt), hospitalized patients (Admit), and patients treated in the ICU during hospitalization, for each week after symptom onset are presented in **C**. The dotted line denotes the cutoff for seroconversion. ELISA measurements were performed in duplicate for each sample and mean OD values are shown. Comparisons between groups were by one-way ANOVA.

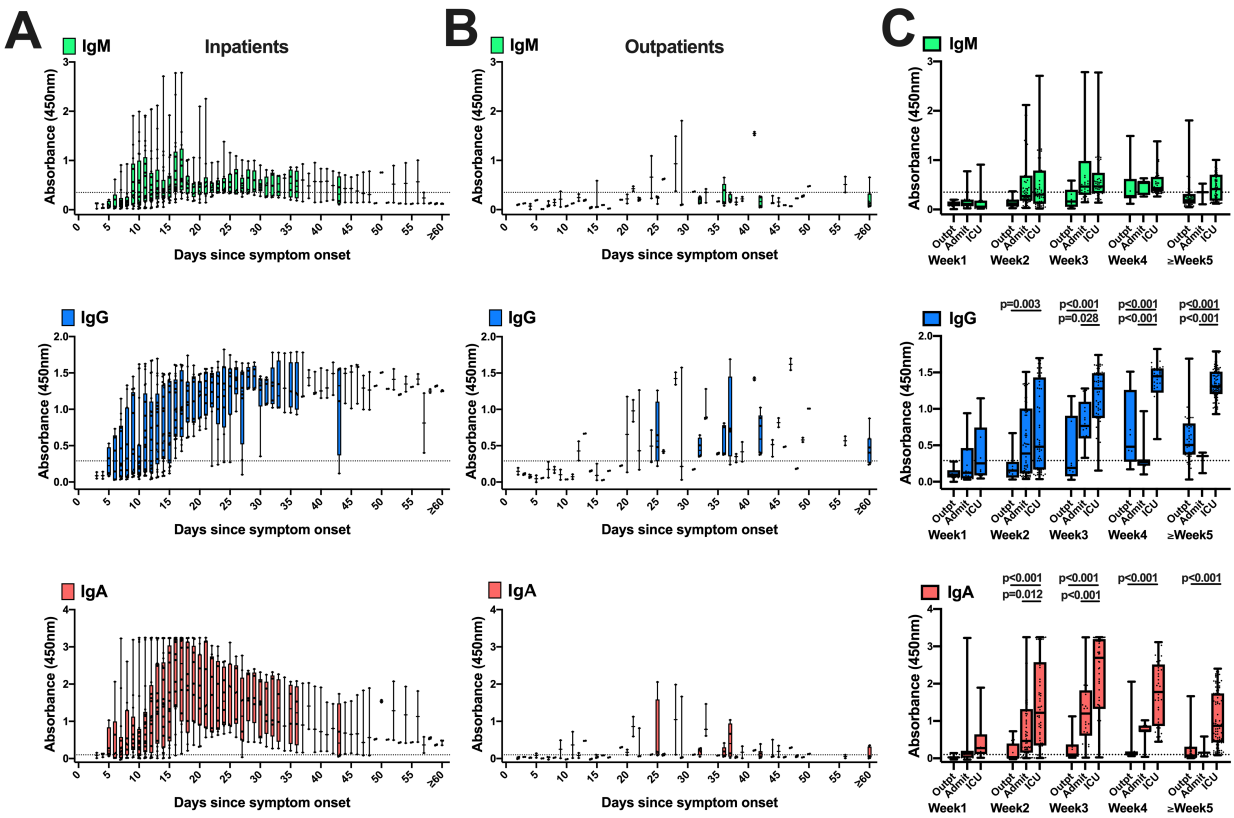

Fig. S3. Development of anti-SARS-CoV-2 N antibody titers in COVID-19 patients.

494 plasma samples from 124 COVID-19 patients were tested for the presence of virus-specific IgM, IgG, and IgA antibodies. ELISA OD_{450} values are plotted separately for samples from inpatients (**A**) and outpatients (**B**). Box-whisker plots show the interquartile range as the box and the minimum and maximum values as the ends of the whiskers. Data stratified by outpatients (Outpt), hospitalized patients (Admit), and patients treated in the ICU during hospitalization, for each week after symptom onset are presented in **C**. The dotted line denotes the cutoff for seroconversion. ELISA measurements were performed in duplicate for each sample and mean OD values are shown. Comparisons between groups were by one-way ANOVA.

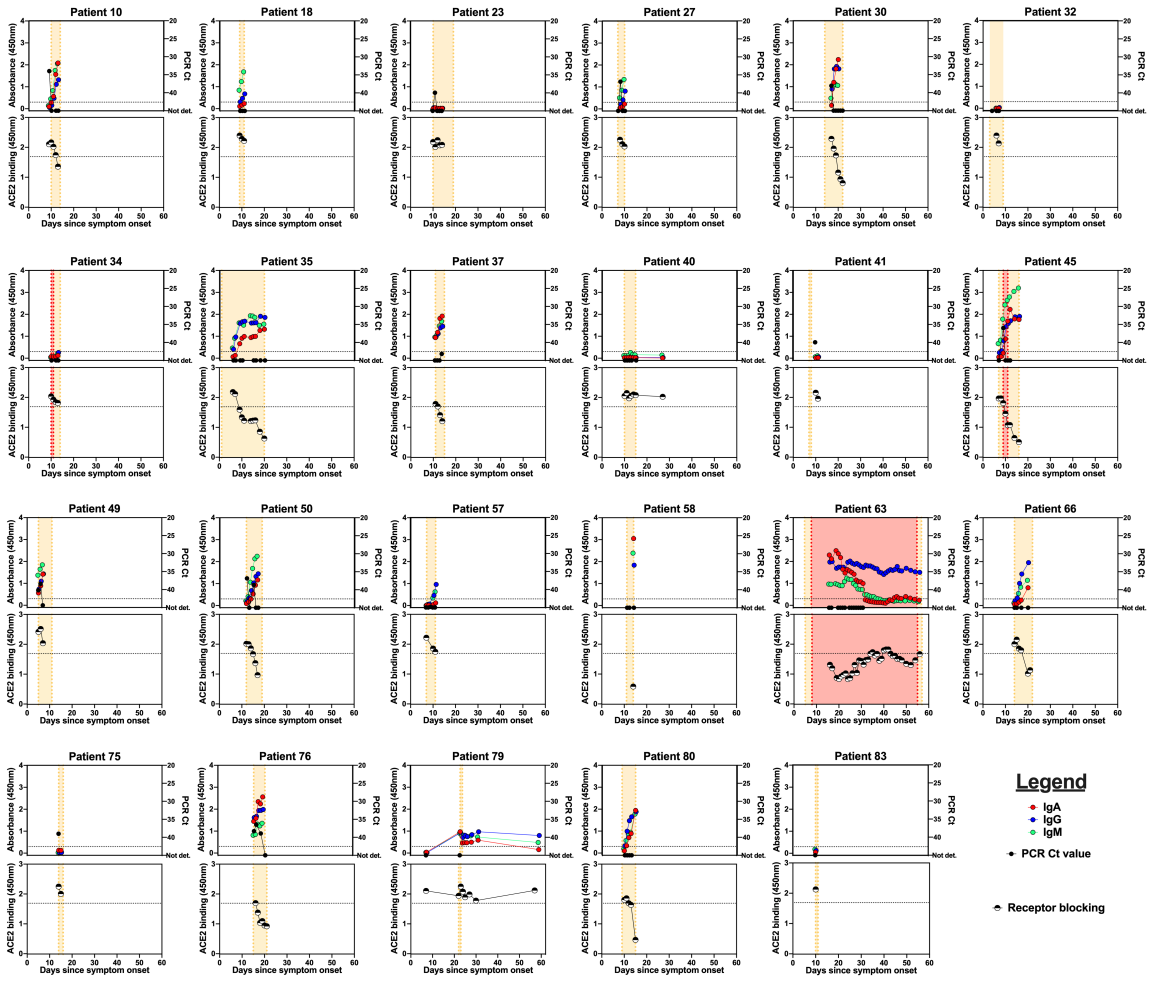

Fig. S4. Anti-SARS-CoV-2 RBD antibodies are correlated with a decrease in viral RNAemia.

Dots with connecting lines in the upper panels illustrate the development of anti-SARS-CoV-2 RBD IgA, IgG, and IgM antibodies (left y-axis) and the rRT-PCR cycle thresholds (black, right y-axis) measured in plasma samples from each individual patient not shown in the main body of this manuscript. Dots with connecting lines in the lower panels illustrate the presence or absence of antibodies preventing binding of ACE2 to RBD in a competition ELISA. The dotted line in each plot denotes the cutoff for seroconversion. Orange shading indicates time admitted to the Stanford hospital, red shading represents the time patients were treated in the ICU. No plots are shown for outpatients nor for one of the 2 inpatients for whom no information on the day of symptom onset was available.

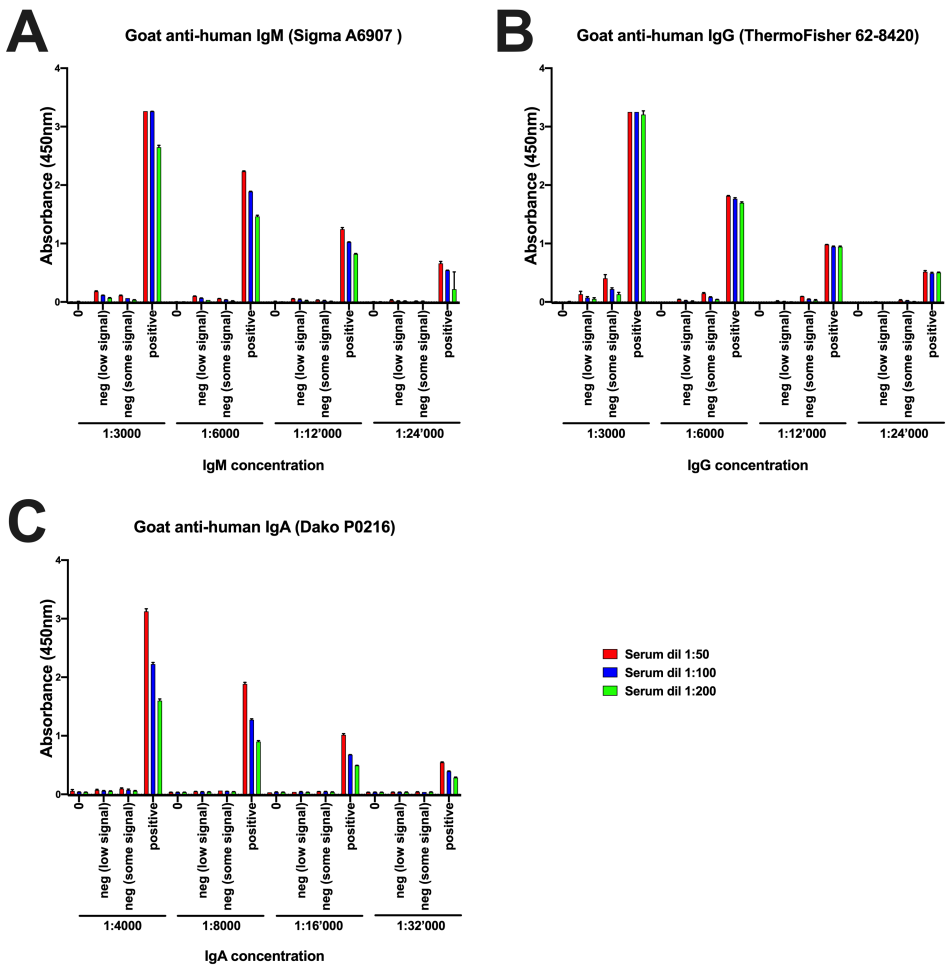

Fig. S5. Checkerboard titration for serological RBD ELISA.

Plasma dilutions (1:50 in red, 1:100 in blue, and 1:200 in green) of two historical negative control samples (one giving a low background and one giving a higher background signal in initial ELISAs), a positive control sample from a COVID-19 patient and a blank control consisting of 1% milk in PBS-T were tested together with different concentrations of anti-IgM, anti-IgG, and anti-IgA secondary antibodies in the SARS-CoV-2 Spike RBD protein ELISA. For an optimal signal to noise ratio, a 1:100 plasma dilution was selected together with a 1:6,000 dilution of IgM **(A)** and IgG **(B)**, and a 1:5,000 dilution of IgA **(C)**.

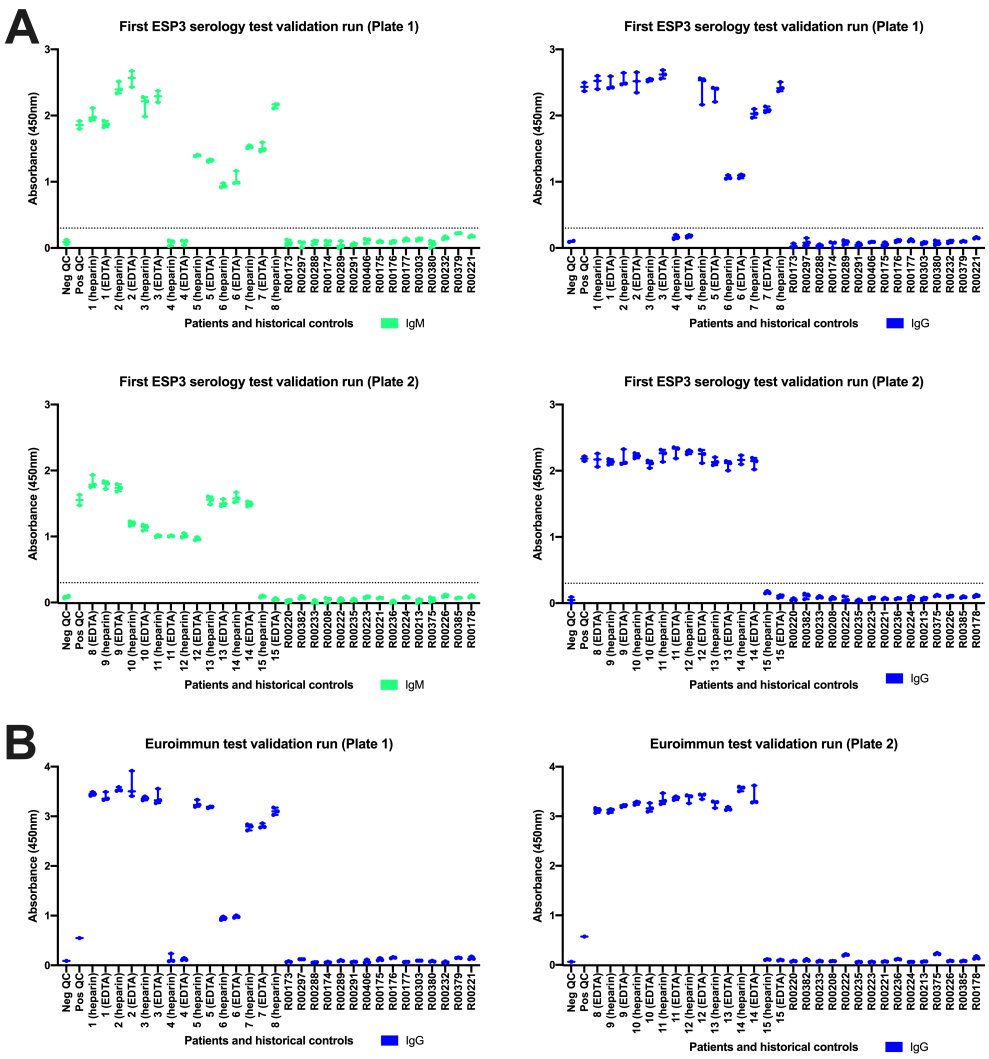

Fig. S6. Validation of the Clinical Lab anti-RBD IgM/G ELISA.

Initial validation of the serological anti-SARS-CoV-2 RBD IgM (green) and IgG (blue) ELISA in the SHC clinical laboratories was done by testing 15 pairs of heparin and EDTA plasma samples from rRT-PCR confirmed SARS-CoV-2-infected patients as well as 30 historical negative control samples collected from healthy blood donors two years prior to the COVID-19 pandemic. OD values for negative controls were used to set a cutoff for seroconversion to 0.3 based on mean values and addition of 3 standard deviations. IgM and IgG-negative samples from known SARS-CoV-2-infected patients in sample pairs 4 and 15 were collected at relatively early

time points post-onset of symptoms (days 14 and 16, respectively). Measurements for triplicate dilutions run on an ESP 600 (Inova Diagnostics Inc., San Diego, CA) ELISA Platform are shown (**A**). Results for IgG were confirmed by testing the same set of samples on the same ESP 600 instrument with the EUROIMMUN anti-SARS-CoV-2 IgG ELISA kit and protocol (EUROIMMUN: cat. EI 2606-9601 G) (**B**). No IgM ELISA kit is available from EUROIMMUN.

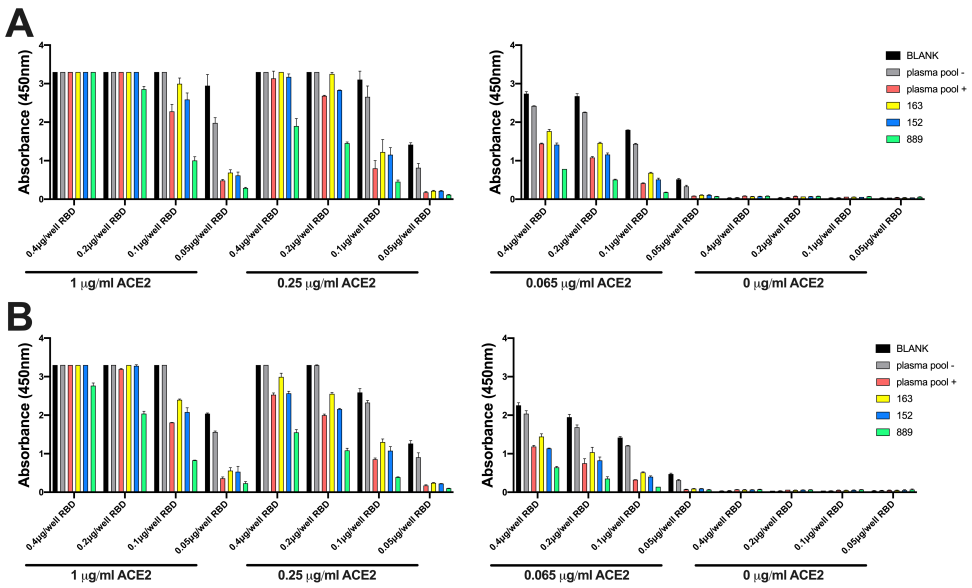

Fig. S7. Checkerboard titration for receptor blocking ELISA.

Plasma dilutions of a historical negative plasma pool sample, a positive plasma pool sample and three positive control samples from COVID-19 patients collected at different timepoints after symptom onset, and a blank control consisting of 1% milk in PBS-T, were tested together with different concentrations of RBD for plate coating, ACE-mFc for competition with plasma antibodies of binding to RBD, and anti-mouse Fc secondary antibody. For an optimal signal to noise ratio, a 1:50 plasma dilution was selected together with coating 0.1 µg/well RBD, with adding 0.25 µg/ml ACE2 and detection with a 1:20'000 dilution of anti-mouse IgG.

Supplementary Tables

Table S1: Inpatient seropositivity.

	RBD_IgM	RBD_IgG	RBD_IgA	S1_IgM	S1_IgG	S1_IgA	N_IgM	N_IgG	N_IgA
W1	26.7	30.0	26.7	23.3	33.3	26.7	13.3	36.7	66.7
W2	69.4	73.4	66.9	70.2	75.0	58.9	43.1	56.9	81.3
W3	90.8	94.3	89.7	88.5	93.1	85.1	67.8	95.4	96.6
W4	98.1	98.1	98.1	100.0	98.1	96.2	78.8	90.4	98.1
W5	100.0	100.0	100.0	88.2	100.0	100.0	73.5	100.0	100.0
W6	76.2	100.0	95.2	66.7	100.0	100.0	66.7	100.0	100.0
W≥7	73.7	97.4	97.4	71.1	97.4	97.4	39.5	97.4	92.1

Percentage of samples positive for anti-SARS-CoV-2 RBD, -S1, and -N (nucleocapsid) IgM, IgG, and IgA relative to the week (W1 to ≥7) after symptom onset.

Table S2: Outpatient and asymptomatic individuals' seropositivity.

	RBD_IgM	RBD_IgG
W1	30.8	40.4
W2	41.9	41.9
W3	62.5	83.3
W4	73.9	87.0
W5	54.5	81.8
W6	46.2	61.5
W≥7	13.3	53.3

Percentage of samples positive for anti-SARS-CoV-2 RBD IgM, and IgG relative to the week (W1 to ≥7) after first SARS-CoV-2 rRT-PCR positive test.